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Elevation of Soluble Fas and Soluble Fas Ligand in Reactive Macrophage Activation Syndromes

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Derailed T-cell activation can give rise to life-threatening macrophage activation, the final common pathway of the different forms of reactive macrophage activation syndromes (rMAS). Besides inappropriate activation of the immune system, impaired termination of immune responses might be another mechanism leading to rMAS. The Fas (CD95)/Fas ligand (CD95 ligand) system functions in turning off immune responses by executing activation-induced cell death (AICD). Soluble Fas (sFas) and Fas ligand (sFasL) can interfere with their corresponding membrane-bound counterparts, qualifying them as potential parameters of impaired immune termination. Hence, sFas and sFasL were analyzed in sera of rMAS patients. We show that soluble Fas/CD95 (sFas) is elevated >2 SD over the mean of controls in all 8 rMAS episodes studied (mean 12.08 ± 6.12 ng/mL, range 3.7–20.2; controls 2.46 ± 0.49, range 1.5–2.9). sFasL was detected during five rMAS episodes (0.70 ± 0.49 ng/mL, range 0.16–1.28; controls all below the limit of detection of 0.1). In addition, both parameters decrease during convalescence, reflecting clinical evolution. In conclusion, sFas seems to be consistently elevated during acute rMAS. sFasL is detected only in a subgroup of our adult rMAS patients extending the recent finding of sFasL elevation in a majority of children with macrophage activation syndromes (Hasegawa et al. Blood 1998;91(8):2793–2799). By interfering with AICD, sFas and sFasL might contribute to the pathogenesis of at least a subset of rMAS. Am. J. Hematol. 64:116–119, 2000. © 2000 Wiley-Liss, Inc.

Key words: non-Langerhans-cell histiocytosis; hemophagocytosis; soluble Fas/CD95; soluble Fas ligand/CD95 ligand; activation-induced cell death

INTRODUCTION

Macrophage activation syndromes are characterized by fever, rash, hepatosplenomegaly splenomegaly, cytopenia, coagulopathy, and frequently signs of capillary leak. Generally, abundant hemophagocytosis can be visualized in bone marrow or other organs of the reticuloendothelial system [1]. Massively elevated serum ferritin levels are a hallmark of disease activity [2]. Though rare, macrophage activation syndromes entail significant lethality. Besides overshooting macrophage activation in the context of malignant histiocytosis and familial hemophagocytic lymphohistiocytosis (FHL) [3], diseases such as infections, neoplasias, and various immunological disorders can lead to reactive macrophage activation syndromes (rMAS) [1,4,5]. The pathophysiology of rMAS remains elusive. Abundant data indicate a pivotal role of T-cells. Derailed Th1-cell activation might emerge due to different stimuli leading to overshooting macrophage activation as the final common pathway [6]. Besides macrophage activation as a consequence of inappropriate T-cell activation, impaired immune response termination might be another potential mechanism leading to rMAS.

Apart from its role in the development of the immune system and cell-mediated cytotoxicity, the Fas receptor/Fas ligand system is a key player in turning off immune responses by executing activation-induced cell death (AICD). Activated T-cells always co-express both Fas and FasL [7]. Both exist in membrane-bound and shed forms. The soluble proteins have been shown to interfere with the signaling of their membrane-bound counterparts

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TABLE I. Underlying Diseases and Laboratory Features of the Patients Studied

#	Age (years)/ gender	Underlying disease	Ferritin[a] (μg/L) p	Ferritin[a] (μg/L) c	sFas[a] (ng/mL) p	sFas[a] (ng/mL) c	sFasL[a] (ng/mL) p	sFasL[a] (ng/mL) c	Hb[b] (g/L)	Tc[b] (G/L)	Lc[b] (G/L)	N[b] (G/L)	LDH[c]	Outcome
1	25/f	JOSD	126,900	6,930	15.1	5.5	1.28	<0.1	70	7	0.7	0.57	25.0	Survival
2	80/f	Legionella pneumonia	9,860	5,100	9.2	4.9	<0.1	<0.1	102	55	14.0	na	na	Death
3	27/f	JOSD/sepsis (Str. mitis)	184,900	10,910	19.4	3.3	1.15	<0.1	70	20	2.1	1.62	25.6	Death
4	62/f	AOSD[d]	57,200	9,000	20.2	2.3	0.53	<0.1	76	24	4.3	0.45	na	Survival
			13,170	910	6.2	4.7	0.16	0.12	86	119	4.2	0.87	2.4	
5	28/f	?	1,530	na	3.7	na	<0.1	na	78	3	8.0	4.88	5.0	Death
6	61/m	?	34,000	na	8.4	na	<0.1	na	90	179	7.5	5.96	2.6	Survival
7	29/m	Endocarditis (Str. mitis)	112,800	na	14.5	na	0.37	na	71	47	4.2	3.30	36.6	Survival

[a]Corresponding peak (p) and convalescence (c) values.
[b]Nadir values for hemoglobin (Hb), thrombocytes (Tc), leucocytes (Lc), and neutrophils (N).
[c]Lactate dehydrogenase maximal fold/upper limit of reference.
[d]Two distinct episodes of rMAS in the same patient; f, female; m, male; JOSD, juvenile-onset Still's disease; AOSD, adult-onset Still's disease; na, not available.

[8,9]. Therefore, sFas and sFasL could contribute to the pathogenesis of rMAS by prolonging the life cycle of activated T-cells. An additional sFasL-pathway may add to the development of rMAS. Recent data point to sFasL as a mediator of inflammation [10,11]. Considering impaired AICD as a potential cause of rMAS, we studied sFas and sFasL serum levels in adults with rMAS.

PATIENTS AND METHODS

Seven adults experiencing eight episodes of rMAS were evaluated (Table I). The rMAS occurred on different clinical backgrounds and were reflected by marked hemophagocytosis and massive hyperferritinemia in all patients. Seven healthy volunteers served as controls.

Sera from 36 blood samples (1 to 9 per episode) drawn during maximal disease activity and in convalescence were stored in aliquots at −80°C immediately after centrifugation. Ferritin levels were analyzed by the Tinaquant® a Ferritin assay (Boehringer-Mannheim AG, Rotkreuz, Switzerland; references 10–160 and 30–300 μg/L for females and males, respectively). sFas and sFasL were determined using commercially available enzyme-linked immunosorbent assays following the manufacturer's protocol (sFas (S) Elisa Kit, and sFas Ligand Elisa Kit, MBL Laboratories Co., Ltd., Nagoya/Japan; limit of detection 0.5 and 0.1 ng/mL, respectively).

RESULTS

sFas values during maximal disease activity were elevated >2 SD over the mean of the control group in all rMAS patients studied (mean 12.08 ± 6.12 ng/mL, range 3.7–20.2; controls 2.46 ± 0.49, individual values: 1.5, 2.3, 2.4, 2.6, 2.7, 2.8, 2.9). sFasL was detected during five episodes of rMAS (0.70 ± 0.49 ng/mL, range 0.16–1.28; controls all below the limit of detection of 0.1 ng/mL) (Table I). Neither sFas nor sFasL peak levels made it possible to predict the outcome. But the available sFasL values show a strong correlation with the corresponding serum ferritin levels (Spearman's rank correlation coefficient 0.6, $P = 0.001$). The correlation ferritin/sFas is close to statistical significance (coefficient 0.37, $P = 0.051$). Serial (≥3 consecutive samples) analysis of five rMAS episodes showed decreasing sFas and sFasL levels in convalescence (Table I). Figure 1 depicts evolution in patient 3, from whom the most samples could be analyzed.

DISCUSSION

We show that sFas is consistently elevated during acute rMAS with a prompt decrease in convalescence. While detected only in a subset of patients, sFasL features a strong correlation with the corresponding ferritin levels which have been shown to be a valuable parameter of disease activity [2]. Recently, Hasegawa et al. reported elevation of sFasL in patients with Diamond-Blackfan anemia and 15 of 19 children with macrophage activation syndromes of whom at least 6 qualified for FHL [12]. Our data extend these findings by demonstrating elevation of sFasL in rMAS of adults and on different clinical backgrounds. Moreover, elevated sFas levels have been reported in a variety of autoimmune diseases [13]. sFasL is detectable in sera of rheumatic disease [13] and leukemia/lymphoma [14] patients. Intriguingly, all these disorders seem to predispose to the development of rMAS [1].

There is much evidence that T-cell dysregulation plays a primary role in the pathogenesis of rMAS. Firstly, elevated (pro)inflammatory cytokines have been reported repeatedly with a preponderance of the Th1 response [6]. Secondly, cyclosporine A and anti-thymocyte globulins seem to be effective in a subset of patients with rMAS

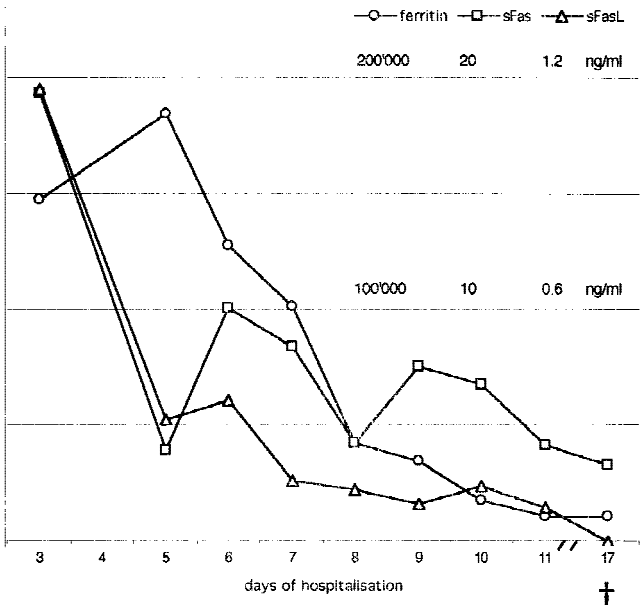

Fig. 1. Evolution of ferritin, sFas and sFasL in patient 3 of whom the most consecutive samples were available. She presented with fever, sore throat, and an urticarial rash of 1 week. Sepsis due to *Streptococcus mitis* was diagnosed at admission originating from a peritonsillar abscess. Although appropriate treatment was installed immediately, a fulminant rMAS developed beginning on day 3 and accompanied by capillary leakage and multiple organ failure. After a period of clinical improvement, fever developed again on day 12 of hospitalization with clinical deterioration but without evidence of a flare up of macrophage activation. The patient died eventually.

[15,16]. Thirdly, in Epstein-Barr virus (EBV) associated rMAS, monoclonal T-cell expansion [17] and clonal rearrangements of T-cell receptors have been shown [18]. Fourthly, patients with T-cell neoplasias are overproportionally prone to develop rMAS. Moreover, various immunological disorders with supposed T-cell involvement predispose to rMAS [1]. Our observations further stress the important role of T-cells in rMAS. Their contribution probably is best reflected by sFas, a T-cell activation parameter found elevated in all episodes of rMAS. Elevated sFasL (and ferritin) might more reflect macrophage involvement. Indeed, sFasL is stored in macrophages and released upon activation [19].

The Fas/FasL system plays a crucial role in turning off immune responses by inducing AICD [7]. Because both sFas [8] and sFasL [9] can interfere with the function of their membrane-bound counterparts, our data support the hypothesis of impaired AICD as a potential mechanism entailing rMAS. Additional support comes from the following findings. Firstly, Fas/FasL dependent cell-mediated cytotoxicity is impaired in FHL [20]. Secondly, in a FHL kindred reduced Tyk2/SHP-1 interaction was demonstrated [21], SHP-1 phosphatase being an effector downstream of Fas in AICD [22]. Although there are discordant data regarding sFas in patients with systemic inflammatory response syndromes [23,24] and sFasL was not detectable at all [23], elevation of sFas and sFasL could alternatively simply reflect shedding of cell surface proteins during severe activation of the immune system.

CONCLUSIONS

sFas seems to be consistently elevated in adults with rMAS, sFasL at least in a subgroup of macrophage activation syndromes of all ages. Both parameters decrease in convalescence. It remains to be demonstrated how far increased circulating levels of sFas and sFasL really correspond with impaired AICD and whether this is indeed a key process in rMAS. A better understanding of the pathogenesis of rMAS could lead to more specific treatment strategies which are needed urgently for a disorder with such a high mortality.

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